CNT 結合アプタマー選択と炭素材料吸着能

CNT-binding aptamer selection and carbon material adsorption capacity

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We proposed a novel CNT/protein/CNT junction for nanodevices. Outer-surface of cage shaped protein could be genetically modified to bind a CNT and make a heterojunction which improves the thermoelectric properties of CNT composites. We used the Ph.D.-12 phage display system for the aptamer SELEX and panning out high-affinity CNT aptamers. We got 223 sequences from 2 different CNT materials, Meijo D and Tuball. We analyzed the hydrophobicity of candidate aptamers with ECS score. In general, the score showed pulsation, suggested aptamers might be displayed the hydrophilic and hydrophobic amino acid on the opposite side of a helix. The DNA-binding protein from starved cells protein (Dps) is member of cage shaped protein which composed of 12 self-assembled 18 kDa subunits that could display N and C terminal at outer shell surface. The aptamer modified Dps protein was produced with protein expression method. These aptamers were linked with Dps using pET20b plasmid with mutagenesis kit. The fusion protein Dps was purified with two steps of HPLC (ion exchange & gel filtration) and confirmed with SDS-PAGE. The adsorption specificity of the purified Dps protein to carbonaceous material was measured by electrochemical impedance spectroscopy (EIS). The measuring solution was PBS, after equilibration, we added 1ug/mL, 3ug/mL, 5ug/mL, 10ug/mL, 30mg/mL Dps to PBS sequentially. The Rct change in low concentration is almost same with C terminal fusion Dps. But at high concentration, it could adsorp more protein on the surface. We can see that aptamer linked Dps has a higher Rct increase ration than wild type Dps with SPE carbon electrodes. We try to produce aptamer with the linker “GGGS” to conect with the Dps. We think that it stabilizes the peptides structure folding between the Dps and aptamer. In the graph, we can see that Y1Nt-Dps has the highest value, even higher than Y1NtC-Dps. Prior to this, we thought that Y1NtC-Dps would have a higher value than Dps with only one end of the aptamer attached. We speculate that compared with the aptamer without linker, the aptamer with linker is more flexible when connecting to the surface of the material, so it is not easy to peel from the surface of the material due to external force. Therefore, the Rct of Y1Nt-Dps is higher than it of Y1NtC.

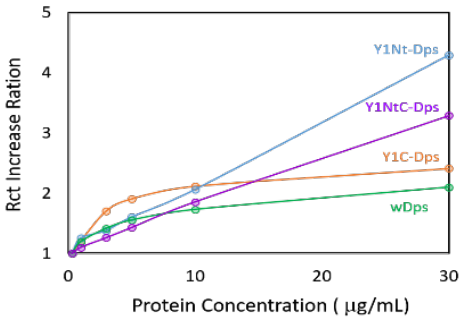

Fig.1 Wild and mutant Dps adsorption dependence on protein concentration

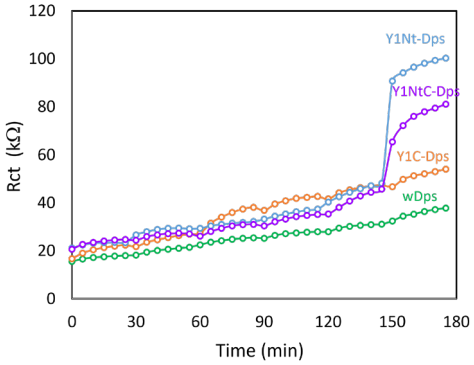

Fig.2 Time course of wild and mutant Dps adsorption.